VESICULATED AXONS IN HAEMAL VESSELS OF AN HOLOTHURIAN, CUCUMARIA FRONDOSA

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There is only fragmentary evidence of neurosecretory activity in echinoderms (Fontaine, 1962; Bullock and Horridge, 1965). A primitive haemal system occurs as a rete in the sea cucumber, *Cucumaria*. In this rete we have found nerve strands containing large dense-cored vesicles in axons which are distributed to the non-striated muscle of the vessel wall.

Specimens were collected from Frenchman Bay, Maine, and kept in running sea water at 15° C. for a few days. Segments of the haemal rete were ligated to prevent contraction, excised and fixed for electron microscopy. Fixation in a 3.5% glutaraldehyde in phosphate buffer was followed by treatment with 1% osmium tetroxide. Other specimens were fixed in 1% osmium tetroxide with 0.3 *M* sucrose in the fixative, followed by 10% formalin. Tissues were embedded in epoxy resin and sections stained with uranyl acetate and lead citrate.

The wall of the haemal vessel has three layers. The outer layer consists of coelomic epithelial cells, nerve strands and non-striated muscle cells. The intermediate layer consists of a thick, distinctly filamentous basal lamina adjacent to the muscle fibers and a deeper connective tissue consisting of a mucoid matrix containing fibers with a periodicity of 640 Å. The inner layer consists of more or less contiguous cells with processes embedded in the fibrous layer, constituting an endothelium. The "endothelial" cells have fine structural features similar to those of fibroblasts in higher forms.

In the outer layer the coelomic epithelial cells have a columnar peripheral portion containing large oil droplets and, at the level of the nucleus, a prominent Golgi region. The basal portion of these cells extends as two or more tapering processes which pass between the fibrous portions of the muscle cells to reach the basal lamina (Figs. 1, 6, 7 F). In these processes masses of fine parallel filaments fill the terminal portions. Adjacent to the basal lamina the fibrous portions of the muscle cells are oriented in the plane of the basal lamina. Most of the fibers are circumferential but a few are longitudinal. There are cytoplasmic processes from the muscle cell extending into the basal lamina. The nuclei and most of the cytoplasm of the muscle cells are found peripherally among the processes of the coelomic epithelial cells but most of the mitochondria are adjacent to the myofilaments. Rather narrow strands of cytoplasm may connect the nuclear and fibrillar portions of the muscle cells (Fig. 1).

Passing between the numerous processes of the coelomic and muscle cells are groups of axons forming nerve strands (Fig. 1 N). At bifurcations of the rete the strands of axons apparently cross each other (Fig. 4). Nerve strands three to five microns in diameter are common in the wall of the haemal rete (Figs. 2, 3). From

these strands single axons are distributed to the fibrous portions of the muscle cells (Fig. 6). Individual axons vary considerably in cross-sectional area (Fig. 4) and there are frequent expansions which may be rather empty or containing accumulations of mitochondria, lipid and dense-cored vesicles.

Within the axons microtubules are preserved after glutaraldehyde fixation (Fig. 3) and are quite uniformly 260 Å in diameter. A small amount of granular lipid is present. There is a variety of sizes of vesicles in the axoplasm and rows of large (0.2 to 0.3 micron) membrane-bounded bodies with a clear space surrounding a

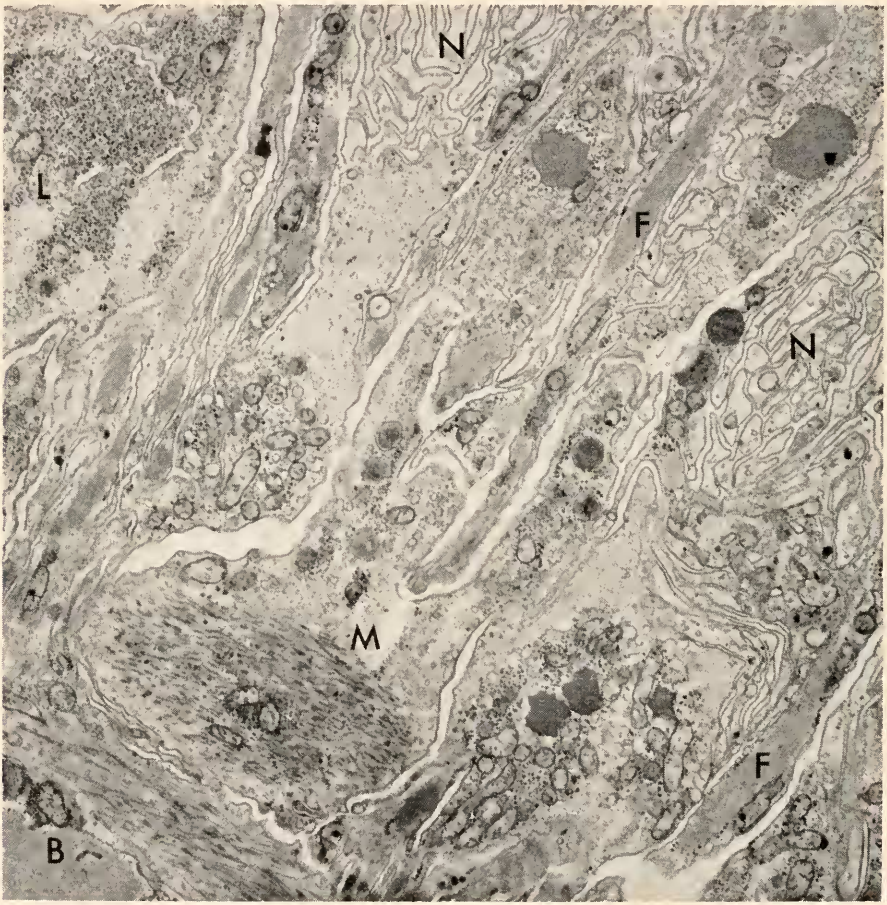


FIGURE 1. Cross-section of the inner portion of the outer layer of the wall of the haemal vessel. Basal lamina, B, at lower left corner and muscle fibers, M, adjacent. The slender dense cell processes extending diagonally across the figure are extensions of coelomic epithelial cells containing filamentous masses, F, and a few mitochondria and lipid granules. A cell with lipid granules, L, is at upper left. In one muscle cell, M, sections of four mitochondria are located among the muscle fibrils and have associated lipid granules. The larger groups of mitochondria in adjacent cells are in the cytoplasmic portions of other muscle cells. Cross-sections of parts of two nerve strands, N, are evident midway in the upper and right hand edges of the figure. Osmic fixation, 7900 ×.

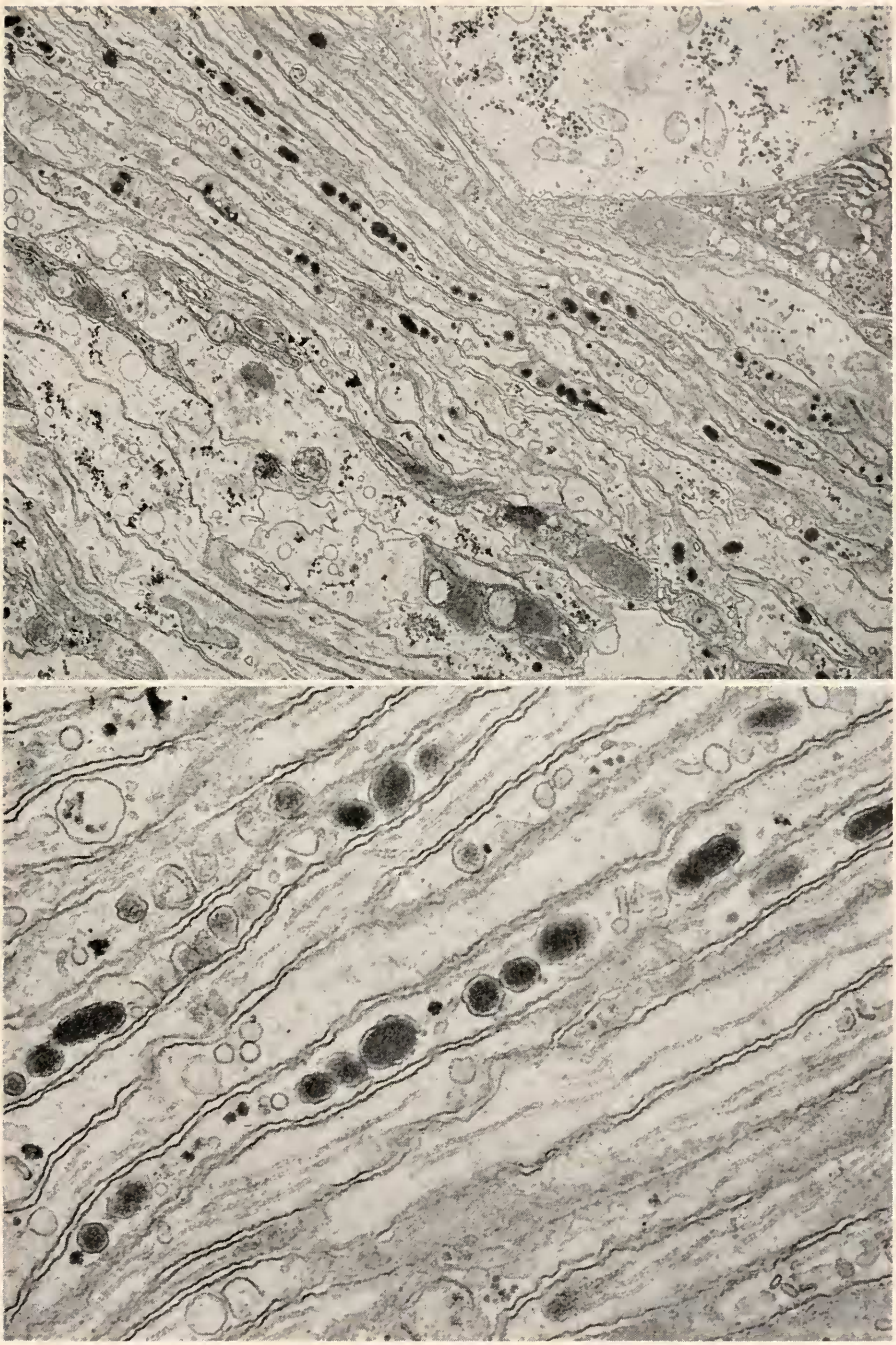


FIGURE 2. Longitudinal section of a nerve strand with several axons running from upper left to lower right. Within the axons are rows of dense vesicles. Glutaraldehyde fixation, 8500 ×.

FIGURE 3. Parallel axons in a nerve strand containing microtubules 260 Å in diameter and membrane-bounded dense vesicles 0.1 to 0.3 micron in diameter. The individual axons approximate 0.5 micron in width. Glutaraldehyde fixation, 27,000 ×.

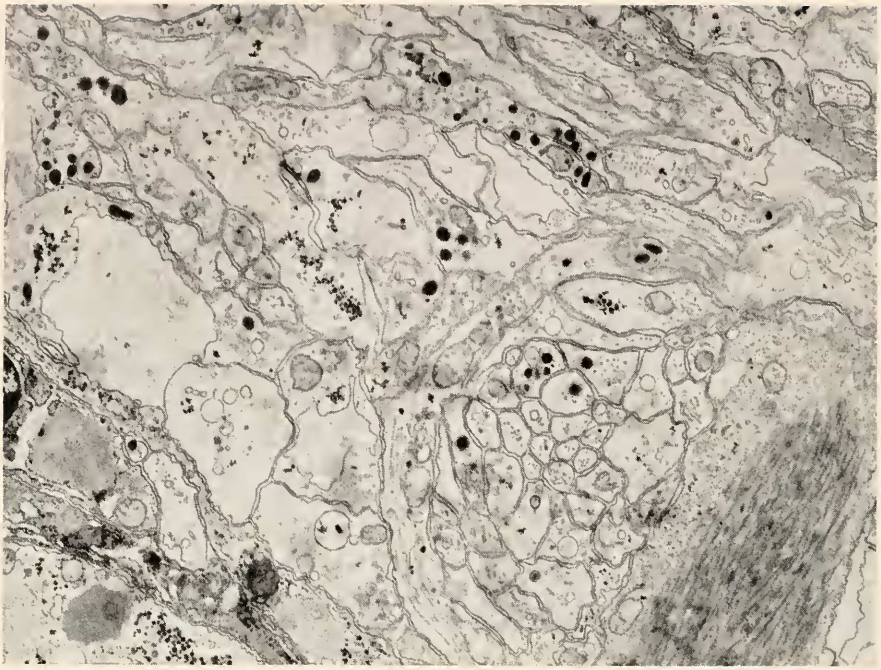


FIGURE 4. Section through a plexus of axons illustrating variations in diameter. Diameters at lower right range from 0.4 to 1.5 microns. Other axons may be identified by the presence of microtubules and specific dense vesicles. The small dense granules in axons are lipid. Glutaraldehyde fixation, 9200 ×.

dense core. These specific vesicles are often elongate and the density of the core varies. In less dense cores the contents can be seen to consist of aggregates of smaller granules or dense vesicles.

No ganglia have been found in the wall of the rete and only occasional single nerve cell bodies along the nerve strands. These cell bodies are distinguished by a folded nucleus, a dilated endoplasmic reticulum which has more ribosomes associated with it in the perinuclear region than in peripheral zones, relatively few mitochondria, a Golgi region, microtubules in the cell processes and in some cases large numbers of vesicles. In one such cell (Fig. 5) the cytoplasm is filled with a variety of heavy-walled vesicles with granular and vesicular contents. In Figure 5 these vesicles are in close association with the Golgi region and give the impression of arising from it. The contents of these vesicles are much less dense than seen in the vesicles found in the axons and some look like multivesiculate bodies. In general, however, the contents are more heterogeneous than seen in multivesiculate bodies and in the elongate forms the contents form denser aggregates. In other cell bodies the dense-cored vesicles have been fewer in number but uniformly more dense, with only a few showing a multivesiculate appearance. Wherever found, the densest particles frequently give some evidence of aggregated composition. It is uncertain whether these bodies are the precursors of those found in the axons but both the heterogeneous and the dense-cored vesicles are confined to the nerve cell

body and axons. No similar vesicles have been found in coelomic epithelial cells, and in hundreds of sections containing muscle cells we have found only a few similar granules in muscle cell cytoplasm.

Sections of cell processes distant from the cell body have been found containing much more uniform populations of a hundred or more dense-cored vesicles. Dilations of the axons in the nerve strands also show accumulations of several vesicles and a few mitochondria and lipid granules.

Axons leaving the nerve strand are often about 0.6 micron in diameter and taper gradually to 0.2 to 0.3 micron at their terminations at the muscle cells. Single dense vesicles may fill the cross-section of the axon termination.

At the muscle cell surface the axons terminate as slightly flattened processes with a distinct intercellular space between the membranes of muscle and nerve cells.

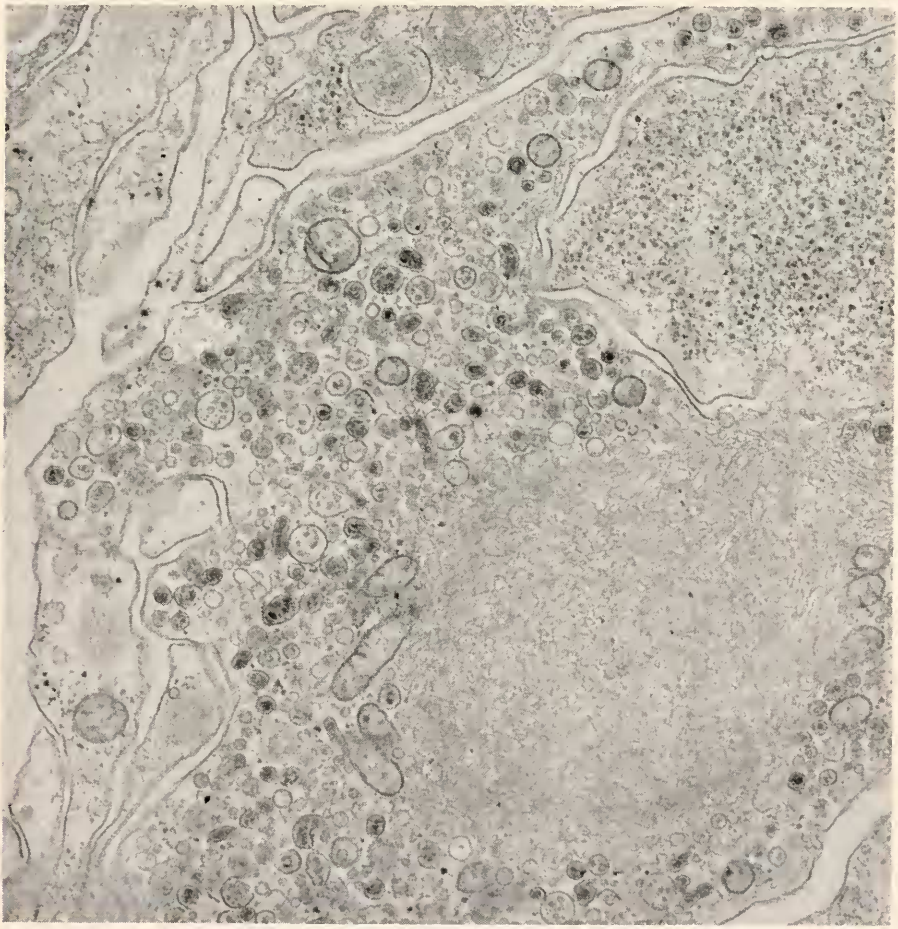


FIGURE 5. Portion of a cell body with a tangential section of the Golgi region surrounded by vesicles of a variety of sizes including some multivesiculate forms. The internal vesicles are of differing densities and degrees of aggregation. Glutaraldehyde fixation, 16,000 ×.

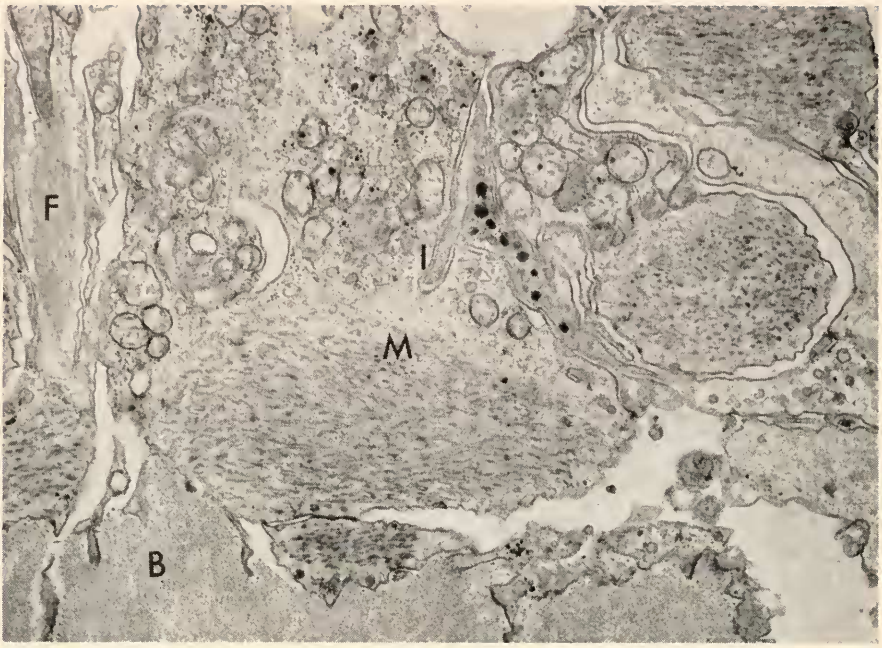


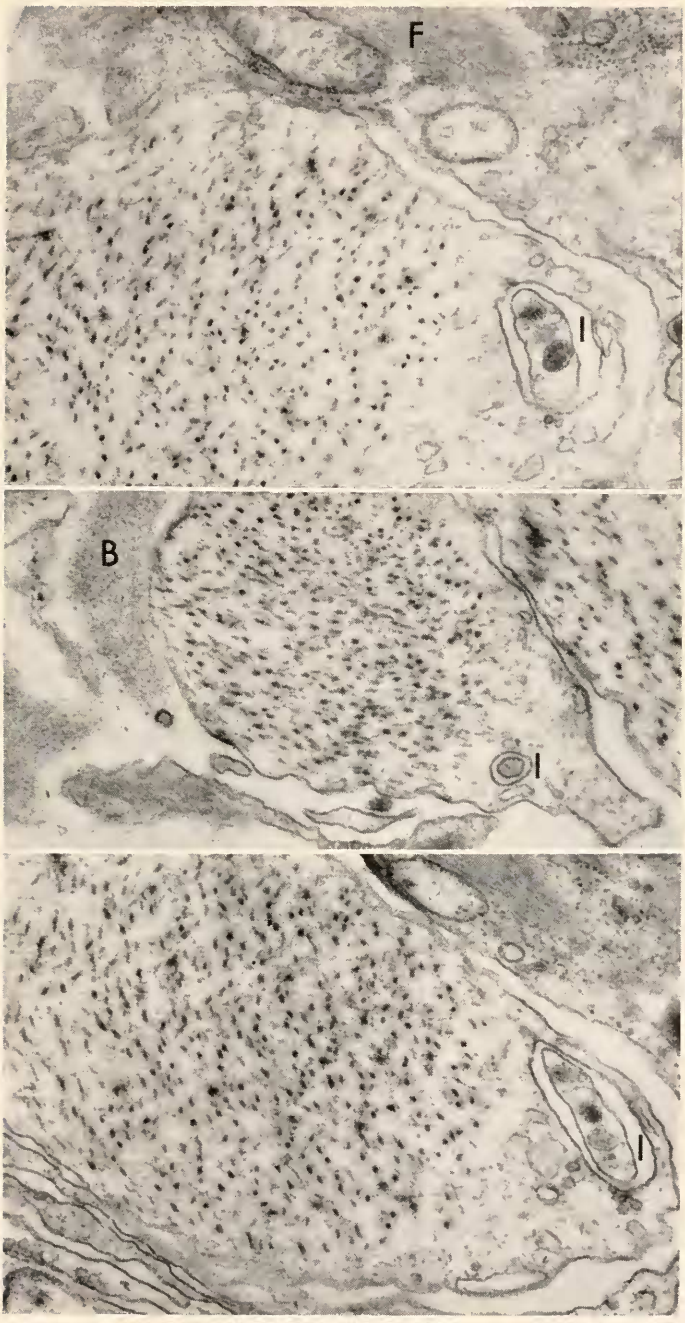
FIGURE 6. Section of a muscle fiber, M, with axon terminals. One axon contains the specific dense vesicles and adjoins the muscle cell membrane at the right. Another, without specific granules, inserts into an invagination, I, of the muscle. Osmic fixation, 11,800 ×.

There is no evident membrane specialization in the regions of approximation. Some of the nerve terminations are in channels of the muscle cell surface with overlapping muscle cell processes. Some axons insert into invaginations of the muscle cell surface but always surrounded by distinct extracellular space (Figs. 6, 7, 8, 9).

DISCUSSION

Cytochemical and electron microscopic evidence for the presence of glycogen is negative in these tissues. The large oil droplets of coelomic epithelial cells and of muscle cells have been observed breaking up into small granules of irregular outline as previously described in the respiratory tree (Doyle and McNiell, 1964) and this lipid may substitute for the glycogen of higher forms. In our preparations the small granules of lipid have irregular outlines and appear as densely stained particles in all of the cells of the outer wall of the haemal rete.

The present evidence on the occurrence of large (0.3 μ) vesicles containing dense aggregates in axons distributed to non-striated muscle cells suggests a possible neurosecretory function in this primitive vascular system. Very little is known of the organization of this part of the nervous system in these organisms. In common with other neurosecretory vesicles the ones present in these axons stain intensely. They appear to arise in the cell body in proximity to the Golgi region. They are distributed along the axons and do not accumulate at the terminals which



FIGURES 7–9. Cross-sections of fibrous portions of muscle cells with tubular invaginations, I, containing axons. Osmic fixation, 35,000 ×.

lack a terminal expansion. The specific vesicles are with rare exceptions confined to the nerve cells and their processes. A specific search for evidence of discharge or transfer of these vesicles has revealed a very few instances of the presence of similar structures in the muscle cell cytoplasm. These very few instances may in fact represent evidence for transfer of vesicles of a transmitter substance but we have no evidence that this is so. In one instance four dense-cored vesicles were seen in the non-fibrous portion of the muscle cell cytoplasm and in another three. In other instances single bodies were observed in the fibrous portion. No evidence has been found of fusion with the cell membrane or discharge from the axon.

Ultrastructural relationships of nerve processes and smooth muscle cells have been described and reviewed recently by Thaemert (1966) while Lever *et al.* (1965) have reported on axon terminals in the arteriolar wall. The occurrence of small specific vesicles has been commonly reported in these studies in higher forms. The close contiguity of nerve and muscle and occurrence of channels in the muscle cell are similar to the relations found in *Cucumaria*. The specific dense vesicles found in *Cucumaria* are much larger structures and their neurosecretory nature remains to be established. Similar structures have been reported in *Hydra* by Lentz and Barrnett (1965).

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Summary

Segments of the primitive haemal rete of the holothurian, *Cucumaria*, were fixed in glutaraldehyde and in osmic acid, embedded in epoxy resin, sectioned for electron microscopy and stained with uranyl acetate and lead citrate. Multifibered nerve strands were found among the epithelial cell processes of the wall of the haemal vessels. Individual axons containing large (0.2 to 0.3 micron) membrane-bounded dense-cored vesicles are distributed to the non-striated muscle cells. The vesicles arise in association with the Golgi region of the neurone and large numbers are found in proximal cell processes. The vesicles containing dense aggregates are distributed along the axons, with a few present at the tapered terminal portions at the muscle cell.

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